



UNIVERSITI PUTRA MALAYSIA

**ISOLATION, CHARACTERISATION AND THERAPEUTIC EFFICACY
OF BACTERIOPHAGE ISOLATES USED AGAINST AVIAN
PATHOGENIC ESCHERICHIA COLI IN BROILER CHICKENS**

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BACTERIOPHAGE ISOLATES USED AGAINST AVIAN PATHOGENIC
ESCHERICHIA COLI IN BROILER CHICKENS**

By

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**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Master of Science**

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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October 2010

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Colibacillosis is one of the main causes of economic losses in the poultry industry worldwide. Although antibiotics have been used to control this infection, the emergence of antibiotic resistant bacteria poses a threat to animal and human health. Therefore, an alternative to antibiotics is urgently required. In the present study, two bacteriophages, namely ØEC1 and ØEC2, which were lytic against avian pathogenic *Escherichia coli* (APEC) O78:K80 and O1:K1 (causative agent of colibacillosis), respectively, were isolated from chickens' fecal samples. These two phages are specific to their target host bacteria and do not lyse other tested bacteria such as *Escherichia coli*, *Salmonella*, *Campylobacter*, *Lactobacillus* and several gastrointestinal microflora.

Examination by transmission electron microscope revealed that ØEC1 and ØEC2 belong to *Podoviridae* and *Tectiviridae* family, respectively. Both phages demonstrated an optimum multiplicity of infection (MOI) of 0.1 to 1. Based on the results of single-step



growth, the latent period of ØEC1 was 25 min with a burst size of 200 particles per infected cell. ØEC2 showed a shorter latent period of 7 min and a burst size of 2-3 particles per infected cell. Under the experimental condition, the highest adsorption rate for ØEC1 was 99.9% and ØEC2 was 82.2%. Both phages demonstrated similar optimum pH (pH 6.0 - pH 9.0) for effective phage lytic activity. The optimum temperature for ØEC1 lytic activity was found to be in the range of 25°C to 41°C while ØEC2 demonstrated the highest lytic activity at 37°C. These results indicated that ØEC1 could be a more virulent phage in comparison to ØEC2. Thus, the efficacy of ØEC1 as therapeutic agent against APEC O78:K80 was evaluated *in vivo* in chickens.

Results from the *in vivo* study showed that treatment with ØEC1 could reduce the mortality rate of *E. coli* challenged birds by 70% during the 3 week experimental period. The body weight of birds from the *E. coli* challenged group treated with ØEC1 was not significantly different from those of negative control groups on day 14 post-infection and was 15.4% higher than that of untreated *E. coli* challenged group on day 21 post-infection. Based on the gross lesion observations for airsacculitis, pericarditis and perihepatitis, the infections were found to be less severe in the treated *E. coli* challenged group. The total viable cell counts of *E. coli* in the lungs of treated *E. coli* challenged birds was approximately 22-fold lower than that of untreated challenged birds at day 1 post-infection. *E. coli* isolation frequency from blood and organs was also found to be lower in treated birds. These results suggested that ØEC1 is effective *in vivo* and could be an alternative to antibiotic to treat colibacillosis in chickens.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**PEMENCILAN, PENCIRIAN DAN KEBERKESANAN TERAPEUTIK
BAKTERIOFAJ TERHADAP “AVIAN PATHOGENIC *ESCHERICHIA COLI*”
DALAM TERNAKAN AYAM**

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“Colibacillosis” adalah salah satu punca utama kerugian ekonomi dalam industri poultri di seluruh dunia. Walaupun antibiotik boleh digunakan untuk mengawal jangkitan ini, kemunculan bakteria yang rintang terhadap antibiotik telah menimbulkan ancaman kepada kesihatan haiwan dan manusia. Oleh yang demikian, alternatif kepada antibiotik adalah sangat diperlukan. Dalam kajian ini, dua bakteriofaj, iaitu ØEC1 dan ØEC2, yang litik terhadap “avian pathogenic *Escherichia coli*” (APEC) O78:K80 dan APEC O1:K1 (agen penyebab kepada “colibacillosis”) telah dipencilkan dari sample bahan buangan ayam. Kedua-dua faj ini adalah spesifik kepada perumah dan tidak menjangkiti beberapa bakteria lain yang dikaji termasuk serotype *Escherichia coli*, *Salmonella*, *Campylobacter*, *Lactobacillus* dan beberapa mikroflora yang dipencil dari sistem pencernaan.

Pemeriksaan dengan menggunakan mikroskop transmisi elektron menunjukkan bahawa ØEC1 dan ØEC2 masing-masing diklasifikasi dalam famili *Podoviridae* dan *Tectiviridae*.



MOI optimum untuk kedua-dua ØEC1 dan ØEC2 adalah dalam lingkungan 0.1 ke 1. Berdasarkan kepada keputusan 'pertumbuhan satu langkah', tempoh latent ØEC1 adalah 25 min dengan saiz letusan sebanyak 200 zarah per bakteria yang dijangkiti. ØEC2 menunjukkan tempoh laten yang lebih pendek iaitu 7 min dan saiz letusan sebanyak 2-3 zarah per bakteria yang dijangkiti. Di bawah keadaan eksperimen, kadar penjerapan tertinggi bagi ØEC1 adalah 99.99% dan ØEC2 adalah 82.2%. Kedua-dua faj menunjukkan pH optimum yang sama (pH 6.0 ke pH 9.0). Suhu optima untuk kegiatan litik yang tertinggi bagi ØEC1 adalah 25°C ke 41°C sedangkan ØEC2 menunjukkan activity litik yang tertinggi pada suhu 37°C. Keputusan ini menunjukkan bahawa ØEC1 adalah lebih virulen berbanding dengan ØEC2. Oleh yang demikian, keberkesanan ØEC1 sebagai agen terapeutik terhadap APEC O78:K80 dikaji secara *in vivo* pada ayam.

Hasil dari kajian *in vivo* menunjukkan bahawa rawatan dengan menggunakan ØEC1 dapat mengurangkan kadar kematian anak ayam yang dijangkiti oleh *E. coli* sebanyak 70% dalam tempoh eksperimen selama 3 minggu. Berat badan anak ayam dari kumpulan anak ayam yang dicabar *E. coli* dan seterusnya dirawat dengan ØEC1 adalah tidak berbeza dari berat badan anak ayam dari kumpulan kawalan negatif pada hari ke-14 dan adalah 15.4% lebih tinggi daripada anak ayam dari kumpulan anak ayam yang dicabar *E. coli* tanpa rawatan pada hari ke-21. Berdasarkan pemerhatian lesi untuk airsacculitis, perikarditis dan perihepatitis, jangkitan pada anak ayam yang dicabar *E. coli* dan diberi rawatan adalah kurang parah. Jumlah bilangan *E. coli* yang didapati dalam peparu anak ayam yang dijangkit dengan rawatan adalah kira-kira 22 kali ganda lebih rendah daripada anak ayam yang dijangkit tanpa rawatan pada hari pertama selepas jangkitan. Frekuensi

pemencilan *E. coli* dari darah dan organ juga didapati lebih rendah pada anak ayam yang dijangkit tanpa rawatan. Keputusan ini mencadangkan bahawa ØEC1 adalah efektif secara *in vivo* dan boleh digunakan sebagai alternatif kepada antibiotik untuk merawat colibacillosis pada ayam.

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I certify that an Examination Committee has met on 25 October 2010 to conduct the final examination of Lau Gee Leng on her Master of Science thesis entitled “Isolation, Characterisation and Therapeutic Efficacy of Bacteriophage Isolates Used against Avian Pathogenic *Escherichia coli* in Broiler Chickens” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P. U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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DECLARATION

I declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or other institutions.

LAU GEE LENG

Date: 25 October 2010



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